

Developmental Cell

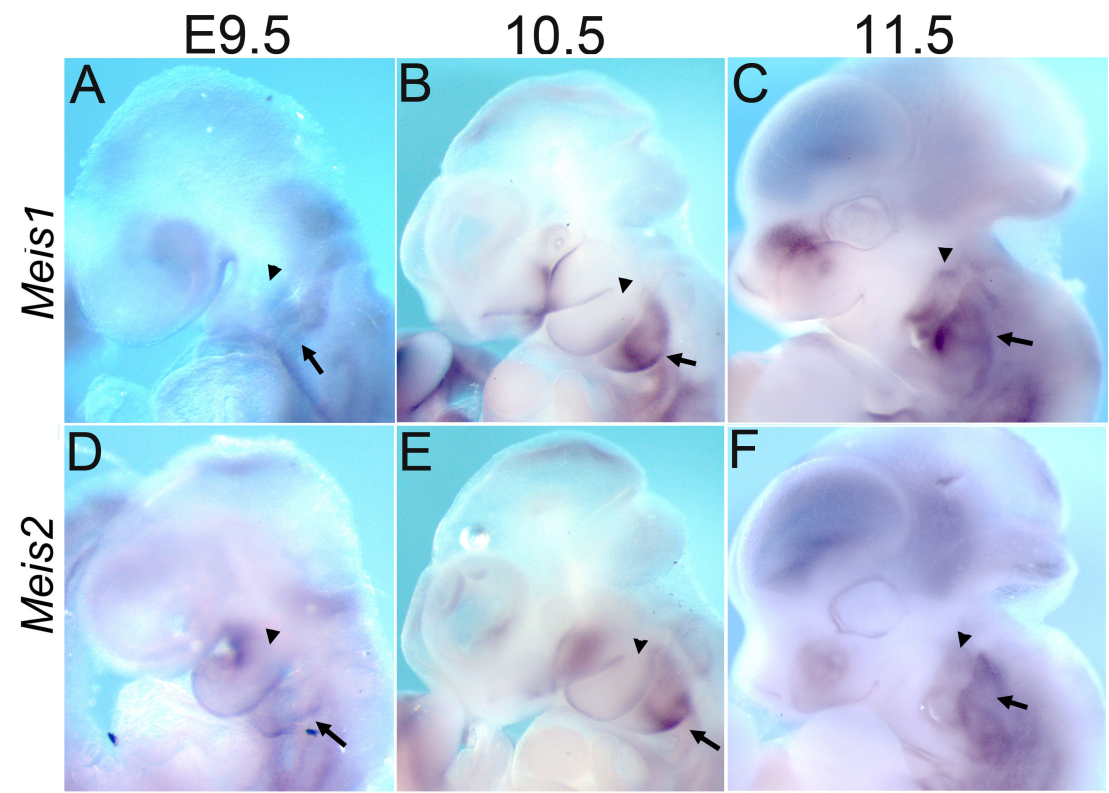
Supplemental Information

## **Hoxa2 Selectively Enhances Meis Binding to Change a Branchial Arch Ground State**

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Supplemental Figures

Figure S1



**Figure S2**

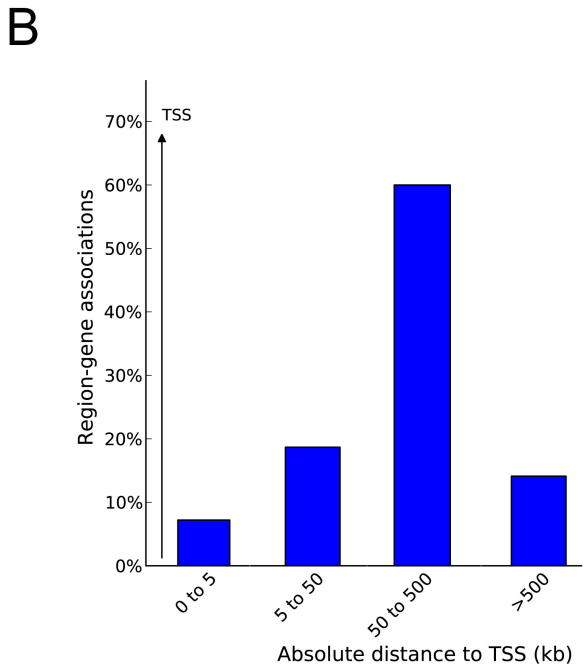
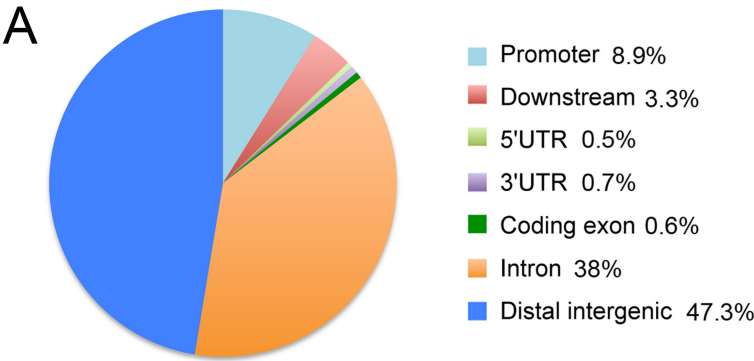


Figure S3

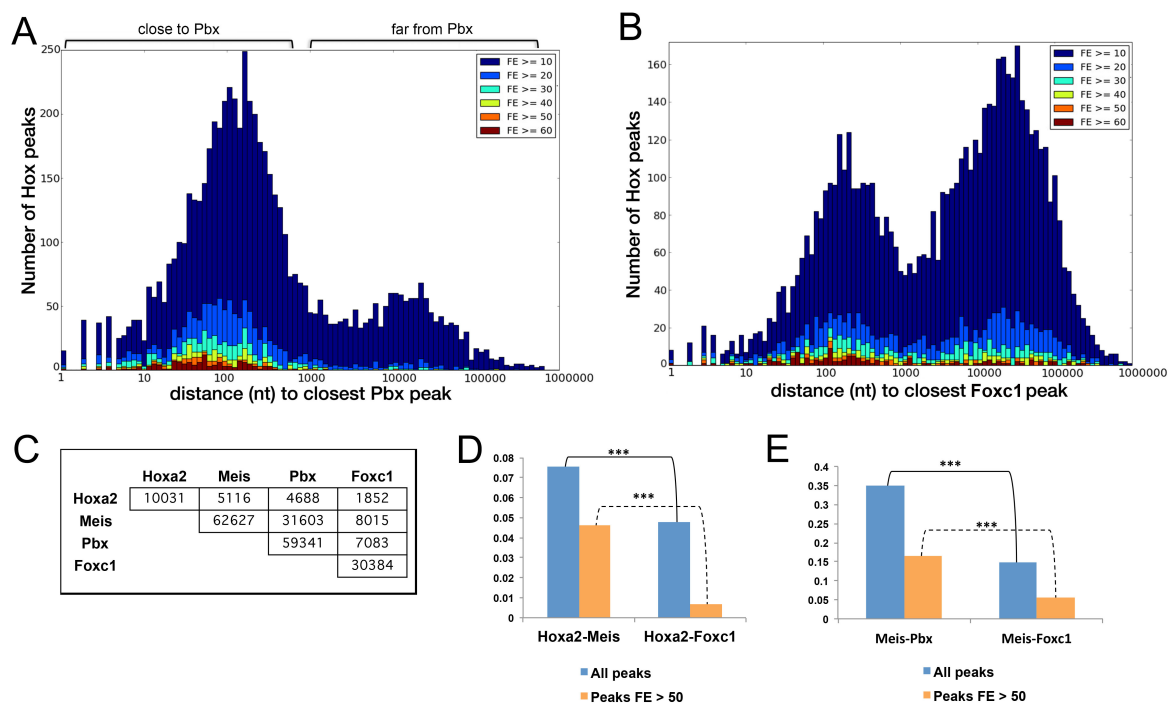


Figure S4

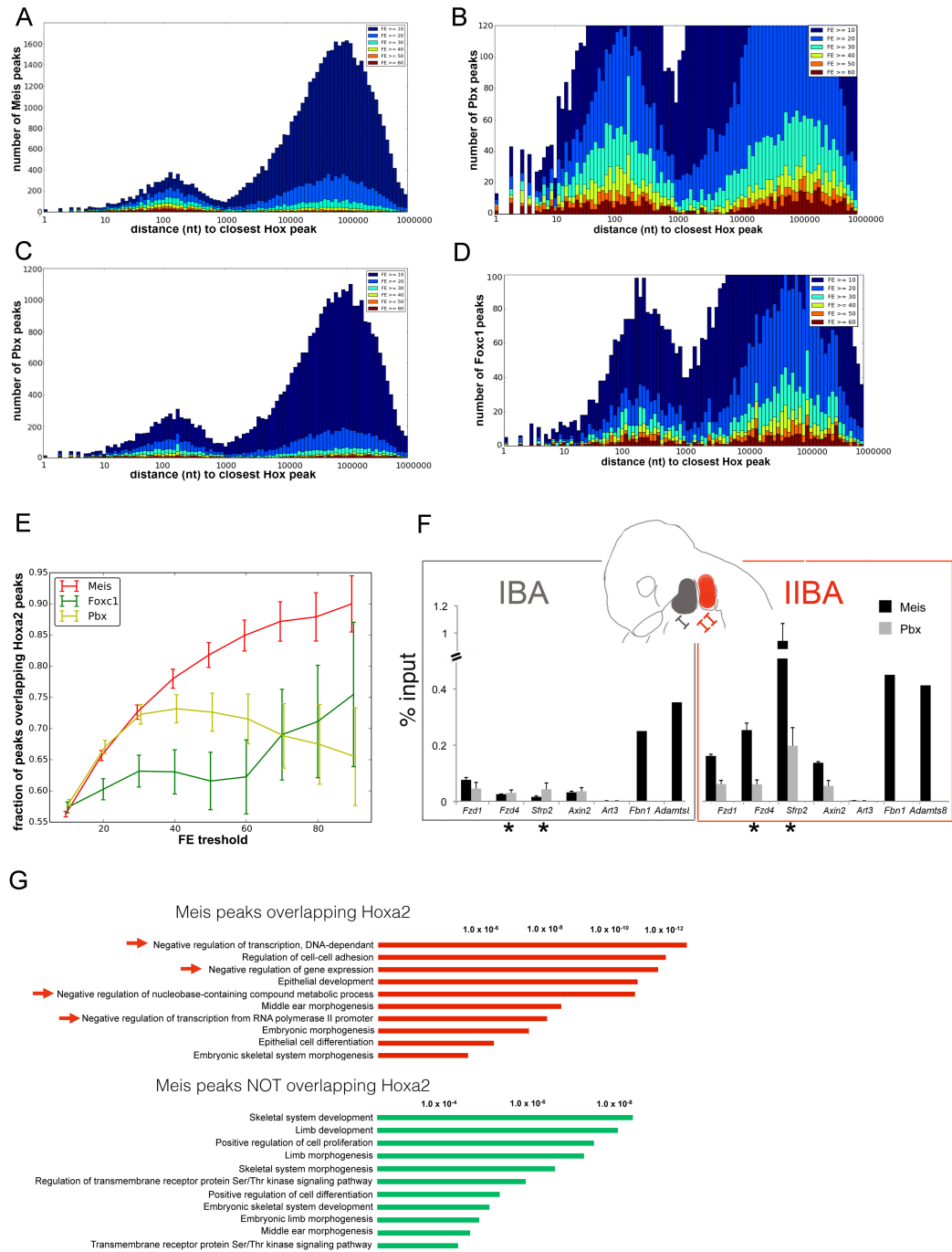
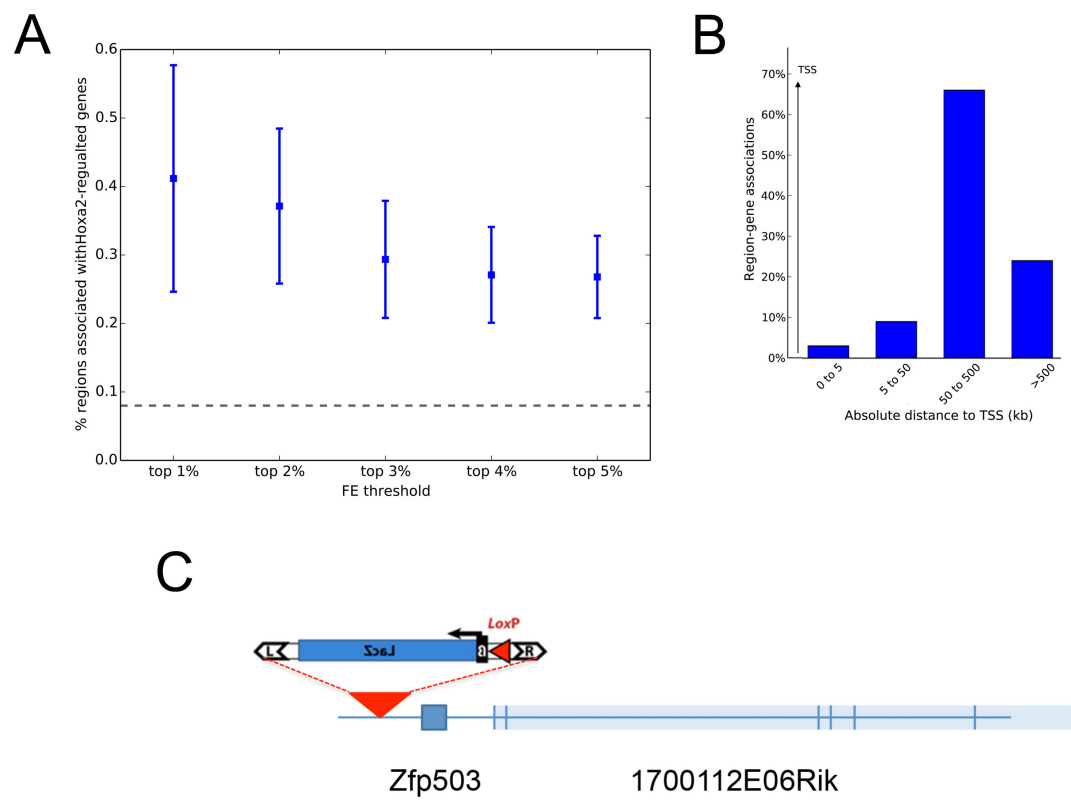


Figure S5



## Supplemental Figures legends

**Figure S1 (related to Figure 1). Expression of *Meis1* and *Meis2* in the craniofacial region.** Whole-mount ISH on wild-type E9.5 (A, D), E10.5 (B, E) and late E11.5 (C, F) mouse embryos, using *Meis1* (A-C) and *Meis2* (D-F) probes. The IIBA (arrows) is one of the main domains of expression of *Meis1* and *Meis2*. Arrowheads indicate the first arch.

**Figure S2 (related to Figure 2). Genome-wide occupancy of Meis TFs in the IIBA.** Distribution of Meis summit regions relative to Reference Sequence (RefSeq) gene structures. The pie charts indicate the proportion of reads for each gene structure; the corresponding numeric values are included. B. Distribution of Meis summits relative to the absolute distance to a transcription start site (TSS).

**Figure S3 (related to Figure 4). Combinatorial binding of Hoxa2, Meis, Pbx and Foxc1** A. Distance of Hoxa2 peaks (IIBA) relative to Pbx peaks. Hoxa2 peaks are binned according to distance to the nearest Pbx peak and labeled according to fold enrichment (FE; high FE is represented by dark red bars and low FE is represented by dark blue bars). B. Distance of Hoxa2 peaks relative to Foxc1 peaks. Hoxa2 peaks are binned according to distance to a nearest Foxc1 peak and labeled according to FE, as above. C. Total number of peaks for Hoxa2, Meis, Pbx and Foxc1 followed by pairwise combinations (number of overlapping 200nt summits). D, E. Significance of pairwise overlaps The co-binding frequency of Hoxa2/Meis is significantly higher than Hoxa2/Foxc1 (D). The co-binding frequency of Meis/Pbx is significantly higher than Meis/Foxc1 (E). Using a binomial test, similar results were observed using the entire ChIP-seq (blue bars) and peaks with FE>50 (orange bars). Asteriks indicate a P value <1E-8.

**Figure S4 (related to Figure 5). Hoxa2 enhances Meis binding.** A. Distance of Meis peaks (IIBA) relative to Hoxa2 peaks (non cropped). Meis peaks are binned according to distance to the nearest Hoxa2 peak and labeled according to fold enrichment (FE; high FE is represented by dark red bars and low FE is represented by dark blue bars). B, C. Distance of Pbx peaks relative to Hox peaks, cropped (B) and uncropped (C). Pbx peaks are binned according to distance to a nearest Hoxa2 peak and labeled according to FE, as above. D. Distance of Foxc1 peaks relative to Hox peaks (cropped). Foxc1 peaks are binned according to distance to a nearest Hoxa2 peak and labeled according to FE, as above. E. Distribution of Meis, Pbx and Foxc1 peaks relative to Hoxa2 binding. The fraction of Meis (red), Pbx (yellow) and Foxc1 (green) peaks close (1kb) to a Hoxa2 peak are plotted with error bars showing 95% confidence region. High Meis peaks ( $FE \geq 40$ ) occur at significantly higher frequencies close to Hoxa2 (1kb) than Pbx or Foxc1 peaks. F. Meis and Pbx occupancy in the IBA (grey panel) and IIBA (red panel) by ChIP-qPCR. *Fzd1*, *Fzd4*, *Sfrp2* and *Axin2* are Hoxa2-bound regions; the IIBA/IBA average enrichment ratios at these regions were 10 and 2.41 for Meis and Pbx, respectively. Regions bound by Meis alone (i.e., *Fbn1*, *Adamts8*, closest Hoxa2 binding >10 kb) showed comparable enrichments when chromatin was extracted from the IBA or the IIBA. *Art3* is a negative control (unbound region). Asterisks indicate regions associated with Hoxa2-regulated genes. Data are presented as average of two independent experiments in duplicate and error bars represent the standard error of the mean. G. Functional categories associated with the top Meis peaks in the IIBA that overlap (red) or do not overlap (green) a Hoxa2 peak (200nt peak summit regions). Meis peaks overlapping Hoxa2 binding display enrichment in the GO term 'negative regulation of transcription' (red arrows), similar to the entire set of top Meis peaks in the IIBA. The length of the bars corresponds to the binomial raw (uncorrected) P-values (x-axis values).



**Figure S5 (Related To Figure 6). Hoxa2 and Meis synergistic binding. A.**

Association of Hoxa2/Meis synergistic binding regions with Hoxa2-regulated genes. Increasing the number of 'synergistic binding events' (by relaxing the FE threshold) results in lower, but still significant associations with Hoxa2-regulated genes, relative to the entire Hoxa2 ChIP-seq (dashed horizontal line at 8%). Top1% (x-axis) indicates the set of top 1% Meis peaks that overlap top1% Hoxa2 peaks; top 2% indicates the set of top 2% Meis peaks that overlap top 2% Hoxa2 peaks, etc. The number of overlapping binding events are : top1%: n= 34; top 2%: n = 70; top 3%: n = 109; top 4%: n = 155; top 5%: n = 209. B. Distribution of Hoxa2/Meis synergistic binding regions relative to the transcription start site (TSS). C. Transposon insertion in the genomic regions containing *Zfp503*. The insertion is located downstream of *Zfp503* (red triangle).

**Supplemental Tables and Legends**

**Table S1 (related to Figure 2).** Meis ChIP-seq dataset in the IIBA

**Table S2 (related to Figure 2).** Meis ChIP-seq dataset in the IBA

**Table S3 (related to Figure 2).** Pbx ChIP-seq dataset in the IIBA

**Table S4 (related to Figure 5). Hoxa2-Meis synergistic binding regions.** List of high confidence Hoxa2-Meis cooperative binding, their associated genes and occurrence of Hox and Meis motifs. Genes dysregulated in *Hoxa2* mutant embryos are in bold.

**Table S5 (related to Experimental procedure).** List of primers used in ChIP-qPCR

<b>Gene</b>	<b>Forwards primer</b>	<b>Reverse primer</b>
<i>Meis1 (1) enhancer</i>	AGATGCCCAGAGAAAGCAAA	GGGGTGTGCATAGAAGGAAA
<i>Meis1 (2) transcript</i>	GCCAATCTTAGCGTCCATTT	TCACTCCGCCATTCTAAACA
<i>Meis2 (1) enhancer</i>	GGGGCGATGCTGTTTATTTA	GGGTGACAGGAATGATGGAT
<i>Meis2 (2) promoter</i>	TCCCGCAGACATCATTGTTA	AAATTGCGCTCGTTCTCCT
<i>Pou6f2 (Donaldson et al. 2012)</i>	CCTGGATTTGACTCGGAAAG	CAGCTGTAACCCGACATTATGA
<i>Itih4 neg (Donaldson et al. 2012)</i>	GAGTCTGCTTGGCTTGAACC	AAGATTGGGCACTTTTTGGA
<i>Hoxa2</i>	TGAGGCGTTCCTTTCTGACT	GGGACCGCGCTACTATTAAA
<i>Fzd1 (Donaldson et al. 2012)</i>	GCCAGAAAATTGTTTGCATAAT	AGAGGGCTGTGGAATTCTGA
<i>Fzd4 (Donaldson et al. 2012)</i>	CTTCCTGGCTCTGCTGAAAG	CAAGGACTCCCATTTACCTCA
<i>Sfrp2 (Donaldson et al. 2012)</i>	TCACCATGACAGGTGGAAAA	CGGAGCTGAAACAGAAGCTC
<i>Axin2 (Donaldson et al. 2012)</i>	TGCTAAAAAGCCGGAAGTA	TCGCAGTCCCTTTGAACTCT
<i>Art3 neg (Donaldson et al. 2012)</i>	GCCAAATTTAGCCAGATCCA	ACGGGGCAACAATTAGCTTT
<i>Fbn1</i>	CCTACCAGCCTGTGTCAATT	TCCTTTTCCCAAGTCTCCAGT
<i>Adamts8</i>	CAGCCTCCACTTAGCCAATT	GGCAACATTTCTGGCACTTTC

<i>Zfp503</i>	TCCTGGTCTGTTTAATGTTTGCT	GCCATCATCTAAAGCACAGAGG
<i>Zfp703</i>	GGCTCCGATGGCTGTAATAA	TGCATTTGCAAAGACGGCTA
<i>Wnt5a</i>	AAGACCTCCTTGCGATCTGA	AAACACCAGGGGCAATCATA
<i>Cxcl12</i>	CACTGATTGGAACCAGATGGG	GCTTGCCAAGTGTGTCGCTAA
<i>Neg Chr17</i>	TGAAGTACAGAAGCGAATGAAGG	TTTGCATGCCAGTGATACTTATC

## Supplemental Experimental procedures

### ISH probes

Probes used for *in situ* hybridization were *Meis1*, *Zfp503* (gifts from Dorothea Schulte and Carol Wicking), *Meis2* (targeting *Meis2* 3'UTR), *Zfp703* (Slorach et al., 2011) for mouse and *dlx2a* (Akimenko et al., 1994) and *hoxa2b* (Prince and Lumsden, 1994; Prince et al., 1998) for zebrafish.

### Antisense morpholino oligonucleotides injections

Antisense morpholino (MO) oligonucleotides were designed to the translation start sites of *meis1* (5'- TATCTTCGTACCTCTGCGCCATCGC-3'), *meis2a* (5'- CTCATCGTACCTTTGCGCCATCAGC -3'), *meis3* (5'- AACTCCTCATACCTCTTATCCATGC-3'), and *meis4.1a* (5'- AGATCCTCGTACCGTTGCGCCATGA-3'), with a corresponding 5bp mismatch MO designed to *meis4.1a* (5'- AGtTgCTCcTACcTTcCGCCATGA-3') (Gene Tools, LLC). A combination of four antisense morpholinos (MOs) was injected, each with a concentration of 0.7 mM (final combined concentration of 2.8 mM) in 1nl total volume. A corresponding 2.8 mM mismatch control MO was also used.